Supporting Information for

A near-infrared fluorescent probe for monitoring viscosity in living cells, zebrafish and mice

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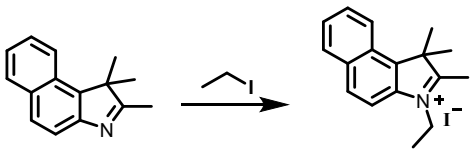
Experimental

Materials and Methods

Common reagents or materials were obtained from commercial suppliers without further purification except as otherwise noted. All experiments used ultra-pure water. The pH measurements were performed with PHS-3E pH meter. Viscosity value was measured by a NDJ-8S rotary viscometer. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope. 1H and 13C NMR spectra were measured on an AVANCE III HD500 digital NMR spectrometer, using tetramethylsilane (TMS) as internal reference. High resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T. Small Animal Imaging System (IVIS Lumina III).

Synthesis of compound of ZM-V

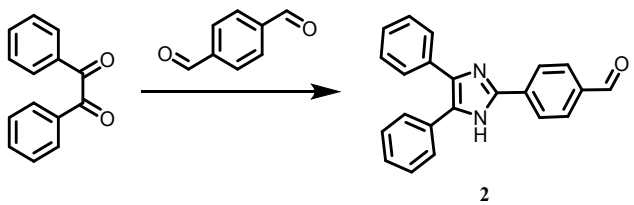
Synthesis of compound 1



1

1,1,2-Trimethyl-1H-benzoindole (1.046 g, 5 mmol) and iodoethane (780 mg, 5 mmol) were dissolved in toluene (5 mL) and allowed to react under stirring at 100 °C for 24 hours. After completion of reaction, the mixture (crude product) was cooled to room temperature and filtered. The residue was washed with petrol ether and dried to give compound **1** as a celadon powder (1.60 g, yield: 88%).

Synthesis of compound 2

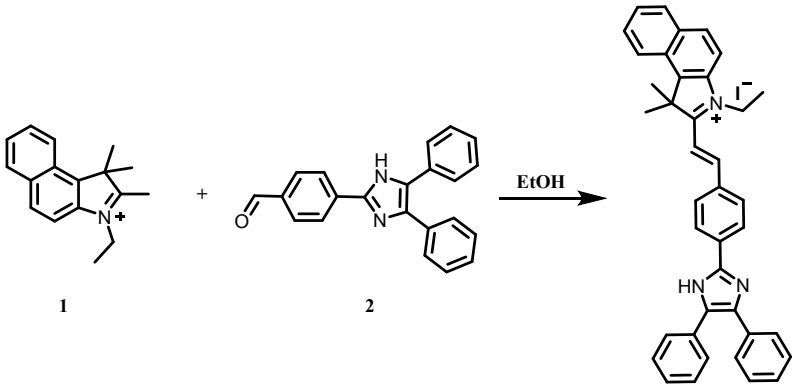


2

Benzil (1.05 g, 5 mmol), 1,4-phthalaldehyde (670 mg, 5 mmol) and ammonium

acetate (3.1 g, 40 mmol) were dissolved in acetic acid, and reacted at 110 °C for 6 h with an inert atmosphere of nitrogen. After completly reacted, the reaction solution was cooled to room temperature, poured into ice water, suction filtered, washed with water and dried in vacuo. The resulting residue was purified by column chromatography on silica gel (petroleum ether to ethyl acetate/petroleum ether =1/10, v/v) to afford the compound **2** as a yellow powder (1.38 g, yield: 85%).

Synthesis of compound ZM-V



Compound **1** (274 mg, 0.75 mmol) and compound **2** (245 mg, 0.75 mmol) were dissolved in 5ml ethanol. Afterward, 50 μL of piperidine was dropped into the mixture, the reaction mixture was at 90℃ for 24 h with an inert atmosphere of nitrogen. The reaction mixture was cooled to room temperature, and then the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel (methylene chloride to methanol/ methylene chloride =1:20, v/v) to afford the compound **ZM-V** as a violet black powder (184 mg, yield:36.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.03 (s, 1H), 8.59 (d, J = 16.3 Hz, 1H), 8.47 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 8.5 Hz, 2H), 8.32 (t, J = 9.1 Hz, 3H), 8.25 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 9.0 Hz, 1H), 7.87 – 7.80 (m, 2H), 7.80 – 7.72 (m, 2H), 7.60 – 7.54 (m, 4H), 7.50 (t, J = 7.5 Hz, 2H), 7.45 (d, J = 7.3 Hz, 1H), 7.35 (t, J = 7.5 Hz, 2H), 7.28 (d, J = 7.4 Hz, 1H), 4.90 (d, J = 7.2 Hz, 2H), 2.07 (s, 6H), 1.56 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 182.29, 152.41, 144.81, 139.27, 138.59, 134.58, 133.73, 131.74, 131.21, 129.75, 127.80, 127.23, 125.88, 123.69, 113.69, 112.23, 54.38, 43.03, 25.93, 14.55. HRMS (ESI): m/z calculated for $C_{39}H_{34}N_3^+$ 544.2747 $[M+N]^+$, found: 544.2762

Spectral measurement

The solvents with different viscosity were obtained by mixing water-glycerol systems in different proportions. Viscosity value was measured by a NDJ-8S rotary viscometer. The solutions of **ZM-V** of different viscosity were prepared by adding the stock solution (1.0 mM) 20 μL to 2 mL of solvent mixture (water-glycerol solvent systems) to obtain the final concentration of the probe **ZM-V** (10 μM). These solutions were sonicated for 30 min to eliminate air bubbles. After standing for 1 hour at a constant temperature, the solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Calculation of fluorescence quantum yield of ZM-V

The fluorescence quantum yields of **ZM-V** were evaluated by using Rhodamine B as a reference standard. The fluorescence quantum yield Φ_s is calculated by the following formula:

$$\Phi_u = \Phi_s \frac{FuAs}{FsAu}\left(\frac{nu}{ns}\right)$$

Where Φ_s is the quantum yield of the sample, F is the area integral value of the corrected fluorescence spectrum, and A and n represent the absorbance and the refractive index of the solvent, respectively. The subscript "u" stands for the unknown to be tested and "s" is the standard.

The Förster-Hoffmann equation

The relationship between the fluorescence emission intensity of the probe **ZM-V** and the solvent viscosity could be formulated by the Förster-Hoffmann equation:

$$\log I = C + x \log \eta$$

Where η is the viscosity, I is the emission intensity, C is a constant, and x is the sensitivity of the probe to viscosity.

Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 96-well tissue culture plate in the presence of 100 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere for overnight and then incubated for 24 h in the

presence of **ZM-V** at different concentrations (0, 1, 2, 5, 10, 15, 20, 25 μM). Then cells were washed with PBS buffer and 100 μL supplemented DMEM medium was added. Subsequently, 10 μL MTT (5 mg/mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 100 μL sodium dodecyl sulfate solution in the water-DMSO mixture. Absorbance of the solution was measured using a microplate reader.

The cell viability (%) = (OD_{sample} -OD_{blank}) / ($OD_{control}$ - OD_{blank}) × 100 %.

Cell culture and fluorescence imaging

HeLa cells were cultured in 90% Dulbecco's Modified Eagle Medium (DMEM, Gibico) supplemented with 10% FBS (Hyclone) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) in an atmosphere of 37 °C and 5% CO_2 The Monensin or Nystatin was first added into the Hela cells in a glass bottom culture dishes (Nest) for 30 min, then they were washed by PBS for three times, after that, the probe (10 μM) was added into the Hela cells treated with Nystatin or Monensin for another 10 min. After washed with PBS for three times, the fluorescence imaging was carried out by a Leica TCS SP8 CARS confocal microscope with a 63 × objective lens. The fluorescence emission of the probe was collected at TRICT channel (600 nm-740 nm), the excitation wavelength was 510 nm.

Fluorescence imaging in living zebrafish

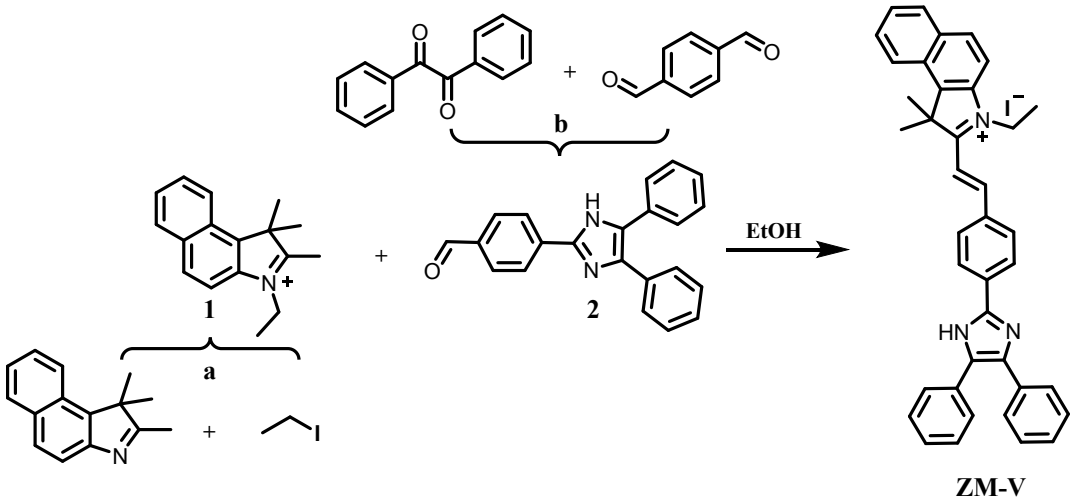
3-day-old zebra fish were transferred into a 30 mm glass culture dishes using a disposable sterilized dropper. The 10 μM Nystatin or Monensin was added for incubated for 30 min, followed by washing away gently. Then probe **ZM-V** (10μM) were put into dishes respectively for another 30 min. After that, the zebrafishes were transferred into new glass bottom dishes for imaging. Prior to the imaging, we adopted 1% agarose gel for immobilization of zebrafishes, and put zebrafishes onto agarose with a little media to ready imaging. Fluorescence images were acquired with Leica TCS SP8 CARS confocal microscope with a 4 × objective lens. The fluorescence emission was collected at TRICT channel (600 nm-740 nm) upon excitation at 510 nm.

Fluorescence imaging in living mice

Female balb/c mice (about 4 weeks aged) were purchased from Guangxi Medical University. The farming system of animals was under standard laboratory conditions. All animal procedures were performed in accordance with the Guidelines for Care and

Use of Laboratory Animals of Guangxi Medical University and approved by the Animal Ethics Committee of Guangxi Medical University (China).

Constructed disease mouse model: The mice were injected with LPS (80 μL, 1 mg ml $^{-1}$) in abdomen to produce viscosity increasing models. After two days, the treated and normal mice were simultaneously utilized for in vivo imaging. Before in vivo imaging, the abdominal fur was removed by an electric shaver and then, the mice were anesthetized by a 4% chloral hydrate aqueous solution (100 μL). The probe **ZM-V** (40 μM) was then injected into the abdominal position of the normal mice and treated. The mice were then imaged by using an in vivo imaging system with an excitation filter of 520 nm and an emission filter of 670 nm.



Scheme S1 Synthesis of probe **ZM-V**. Reaction conditions: (a) Toluene, 100℃, 24 h. (b) Acetic acid, nitrogen atmosphere, 110℃, 6 h.

Table S1 Test viscosity in the varied of the methyl alcohol/glycerol (v/v) mixtures of **ZM-V**.

V %		Viscosity / CP
glycerol	water	
99%	0%	937.48
90%	10%	312.84
80%	20%	111.36
70%	30%	54.74
60%	40%	34.84
50%	50%	25.23
40%	60%	14.57
30%	70%	7.89
20%	80%	3.30
10%	90%	2.63
0%	99%	1.29

Table S2 Selective fluorescence spectrum of **ZM-V** (10 μM) at 666 nm in PBS (pH = 7.4, 0.01 mM) added different analytes. （The concentration of interfering ion was 1 mM.）

Selective fluorescence spectrum			
Analytes	Fl. Intensity (a.u.)	Analytes	Fl. Intensity (a.u.)
Blank	54.92	Glycerol	7947
Ga^{2+}	53.66	Co^{2+}	61.23
Cu^{2+}	90.25	Cu^{+}	37.93
Fe^{2+}	81.94	K^{+}	61.86
Mg^{2+}	58.50	Mn^{2+}	77.72
Na^{+}	54.94	Zn^{2+}	81.26
Hcy	25.38	Cys	42.00
GSH	48.12	HSO_3^{2-}	12.71
H_2O_2	70.86	ClO^{-}	62.82
$NO2^{-}$	55.48	OAc^{-}	60.26
PO_4^{2-}	57.87	SCN^{-}	53.07
CO_3^{2-}	55.15	SO_3^{2-}	12.86
SO_4^{2-}	57.96	$S_2O_3^{2-}$	105.0
Br^{-}	54.07	F^{-}	54.56
Glucose	61.24	—	—

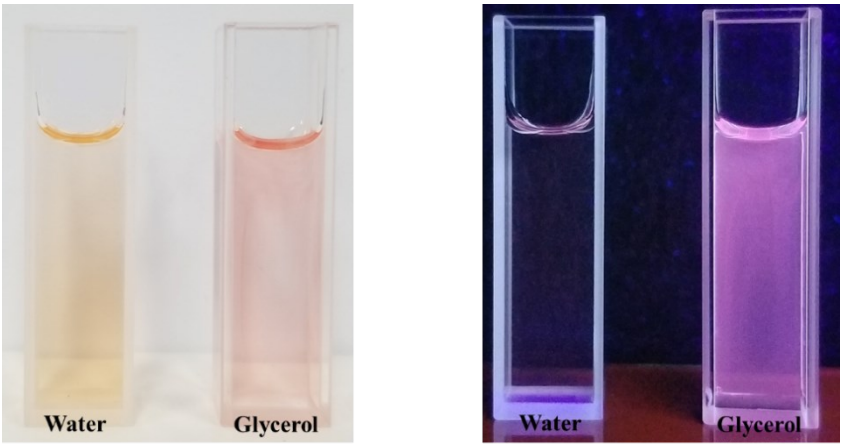


Fig. S1 The color changes of **ZM-V** (10 μM) in water/glycerol solution under ambient light and ultraviolet light.

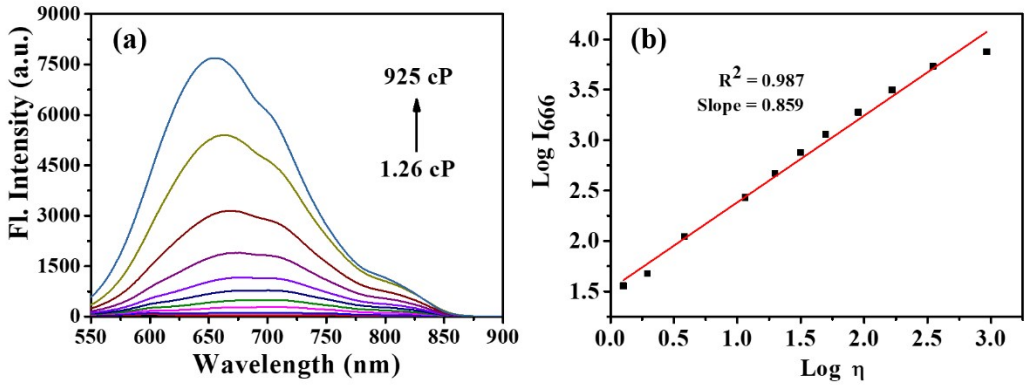


Fig. S2 (a) The fluorescence change of **ZM-V** in water-glycerol solution with different viscosity; (b) The curve is plotted with log (I_{666}) versus log(η).

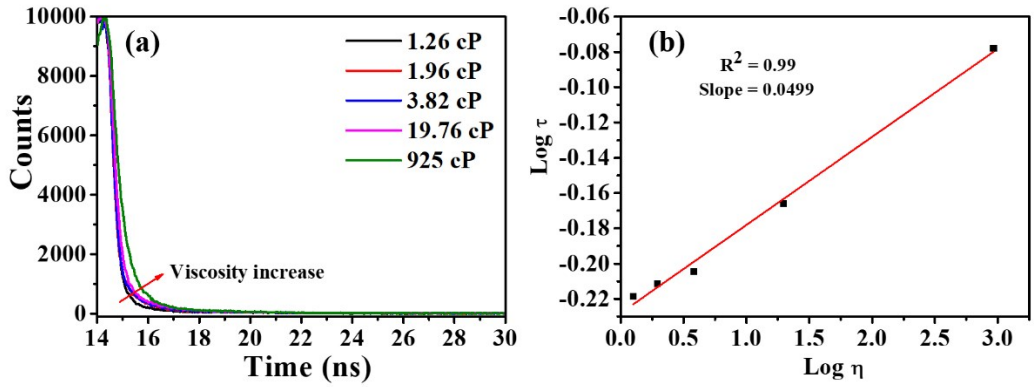


Fig. S3 (a) Fluorescence lifetime spectra of **ZM-V** (10 mM) in water-glycerol solutions with different viscosities. (b) Linear relationship of log τ and log η.

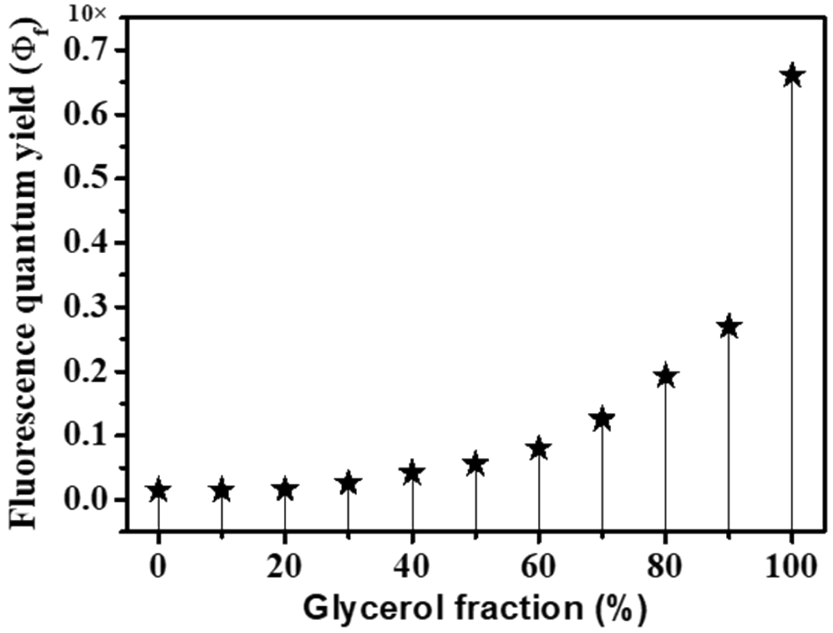


Fig. S4 Fluorescence quantum yield (Φ_f) of **ZM-V** in the water/glycerol (v/v) mixture.

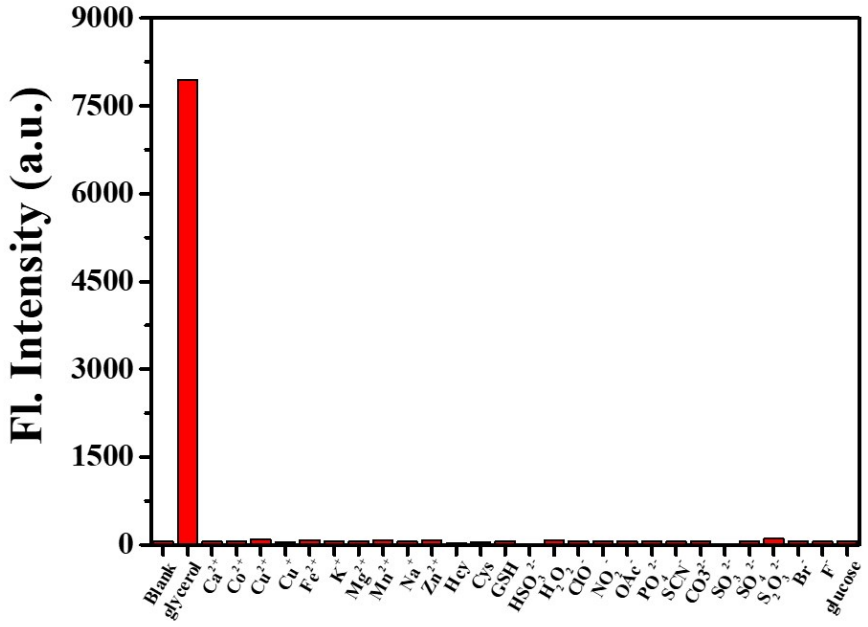


Fig. S5 Selective fluorescence spectrum of ZM-V (10 μM) at 666 nm in PBS (pH = 7.4, 0.01 mM) added with: blank, glycerol, Ca^{2+}, Co^{2+}, Cu^{2+}, Cu^{+}, Fe^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Zn^{2+}, Hcy, Cys, GSH, HSO_3^{2-}, H_2O_2, ClO^{-}, NO^{2-}, OAc^{-}, PO_4^{2-}, SCN^{-}, CO_3^{2-}, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, Br^{-}, F^{-}, and glucose.

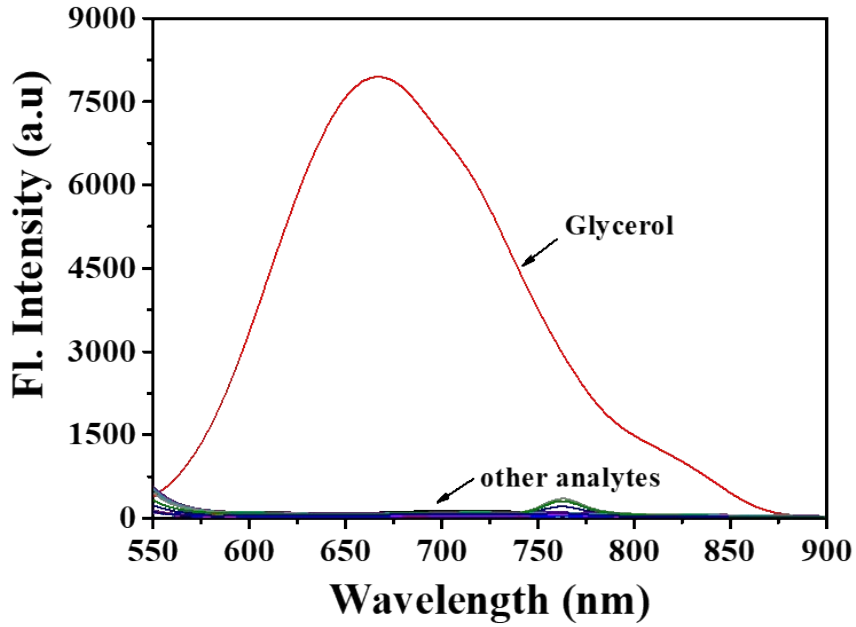


Fig. S6 Selective fluorescence spectrum of **ZM-V** (10 μM) at 666 nm in PBS (pH = 7.4, 0.01 mM) added with different analytes.

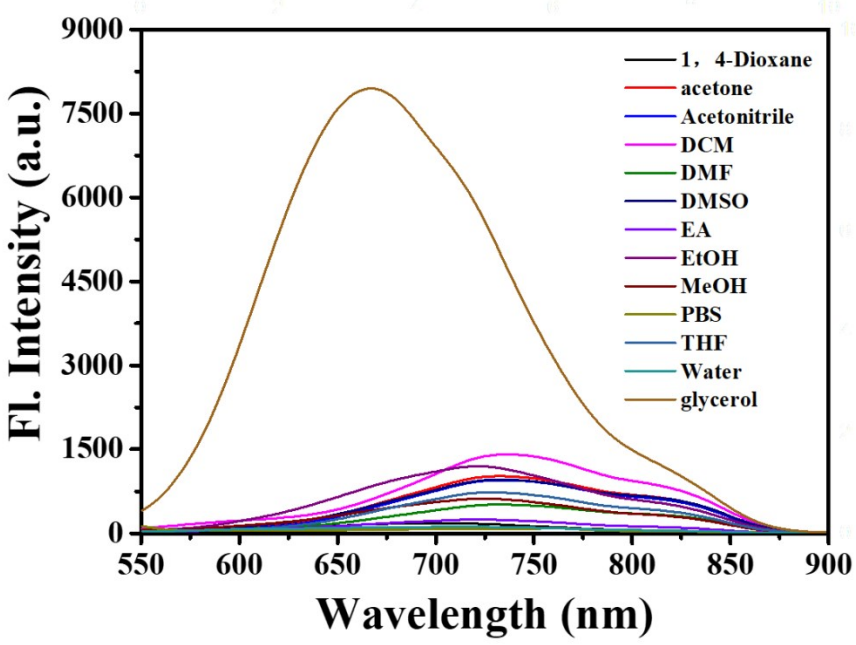


Fig. S7 Fluorescence emission of **ZM-V** in various solvents with different polarities.

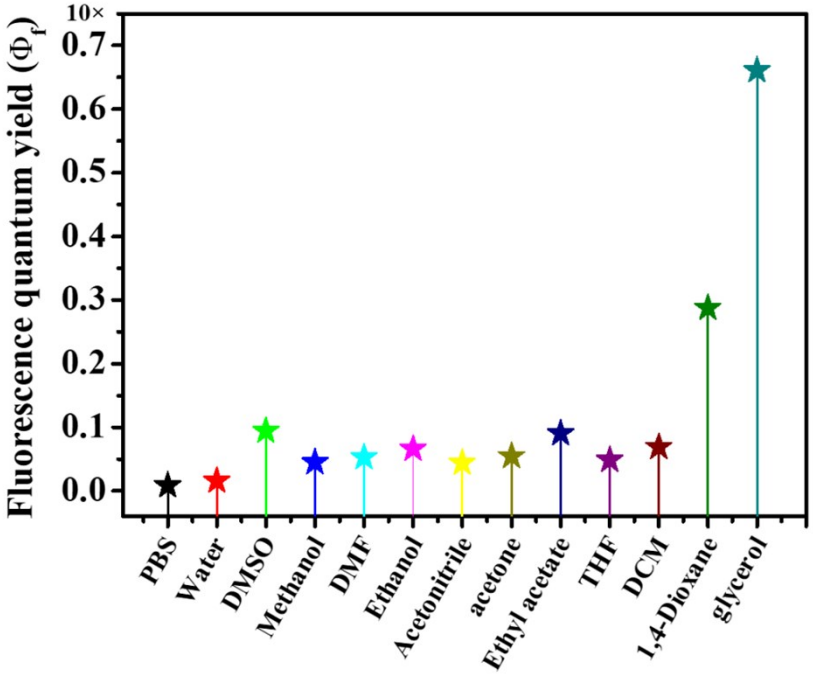


Fig. S8 Fluorescence quantum yield (Φ_f) of **ZM-V** in various solvents with different polarities.

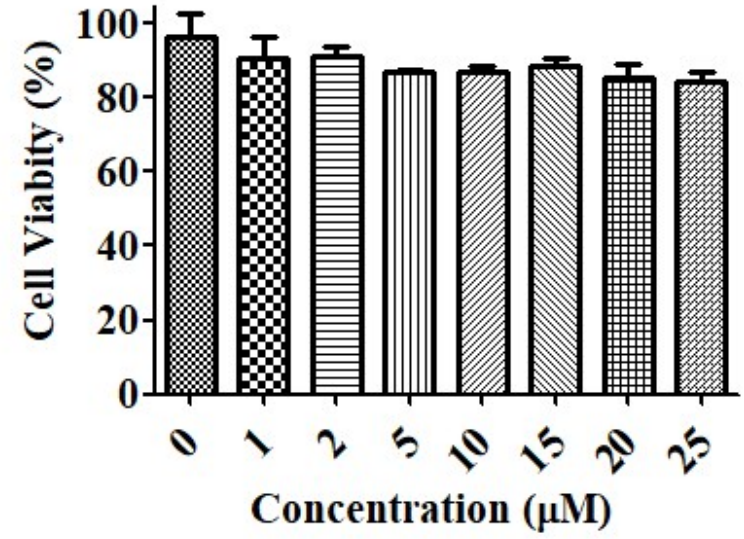


Fig. S9 The cytotoxicity of the probe **ZM-V** in Hela cells.

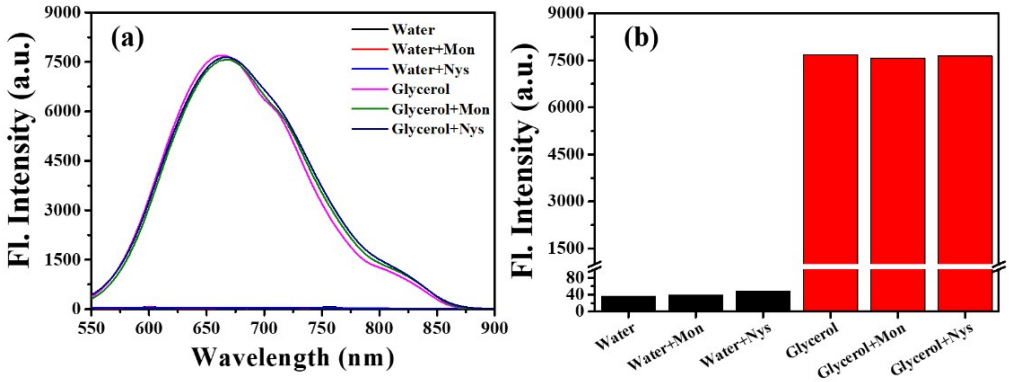


Fig. S10 The fluorescence emission of the probe **ZM-V** in water; water+Mon; water+Nys; glycerol; glycerol+Mon; glycerol+Nys.

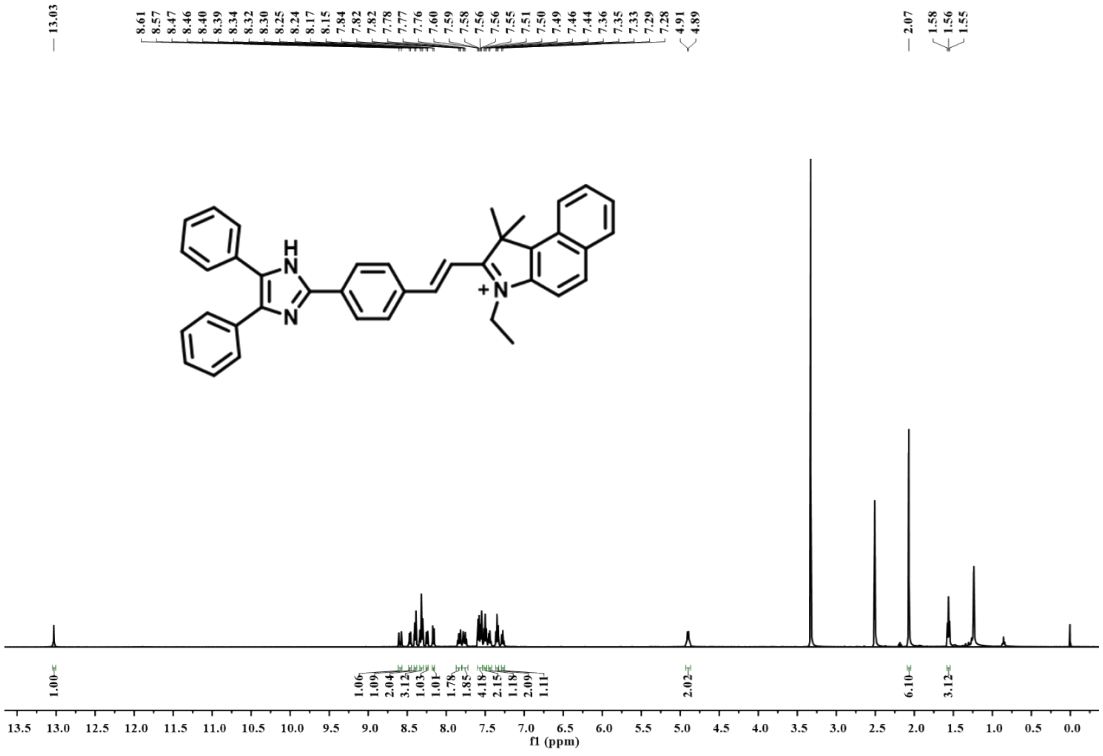


Fig. S11 ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of **ZM-V**.

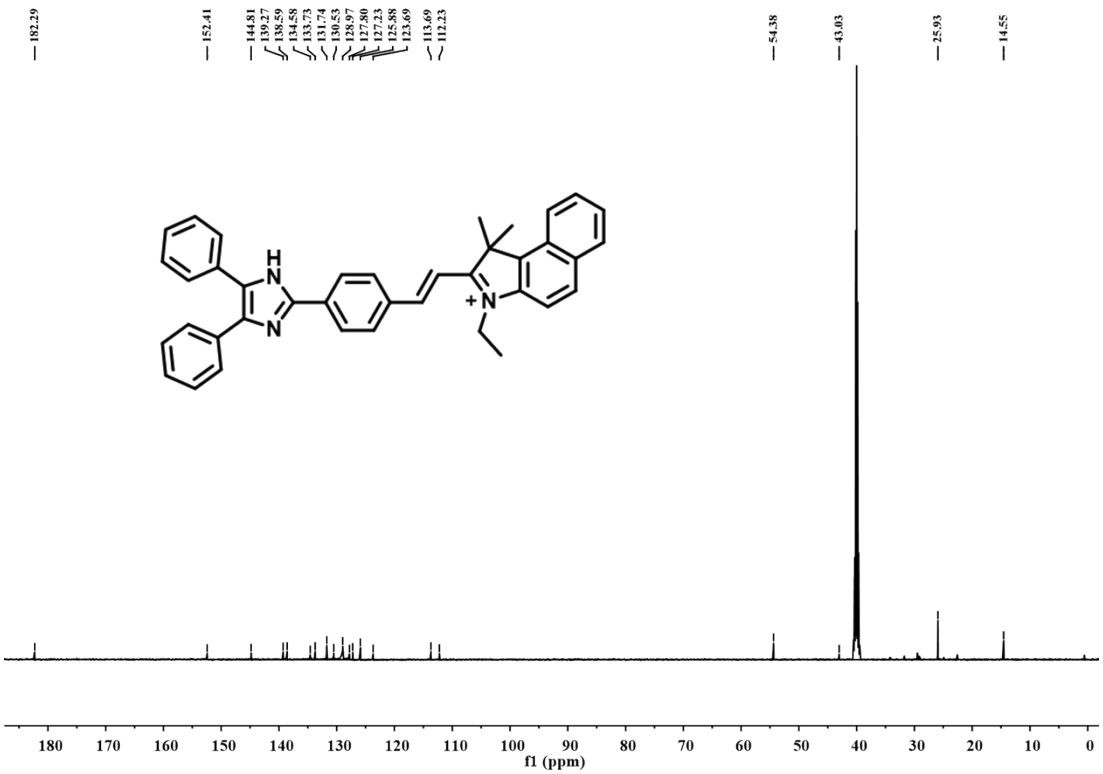


Fig. S12 ^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **ZM-V**.

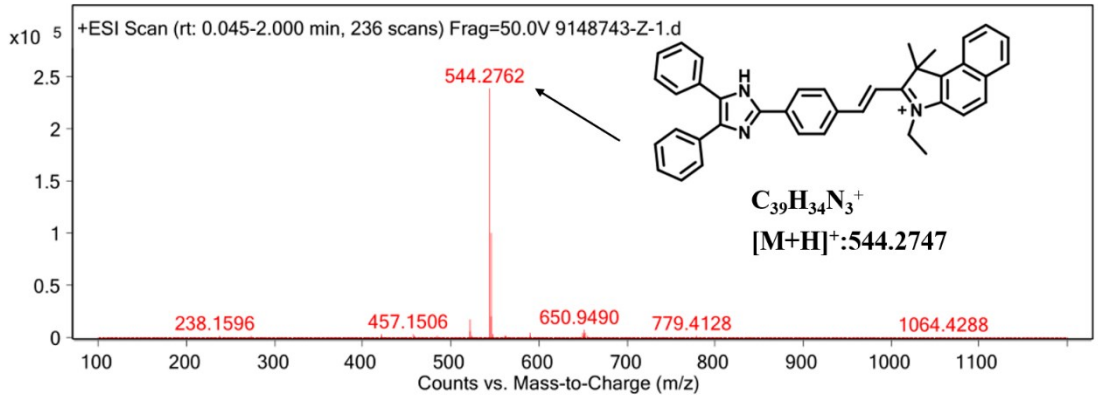


Fig. S13 HRMS (ESI) spectrum of **ZM-V**.